THE JOURNAL OF INVESTIGATIVE DERMATOLOGY
Vol. 49, No. 3

Printed in U.S.A.

A COMPARISON OF PROTEINS IN SERUM AND CANTHARIDIN-PRODUCED BLISTER FLUID AS A FUNCTION OF TIME*

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INTRODUCTION

In recent years increased attention has been given to the formation of blisters and to the constituents of blister fluid. The concentration of protein in blister fluid has been studied using fluid from the pathological blisters of pemphigus and other diseases (1, 2). Lever (1) concludes that the concentration of total protein in the blister fluid of pemphigus vulgaris usually is lower than in the corresponding blood serum. Kandhari (2) differs in reporting that values for the total protein in his work frequently were higher in blister fluid than in the corresponding serums. When the total protein is separated into its several fractions by electrophoresis and immunoelectrophoreses, it is found that the blister fluid reflects the composition of the serum with close correlation of the percentage values (3). Citing these studies, Lever has concluded that blister fluid in pemphigus vulgaris represents serum diluted with interstitial fluid (1).

Since its successful stereospecific synthesis by Storck et al in 1951 (4), cantharidin has been used to produce experimental blisters. Several authors (5, 6) have reported that the protein concentration of cantharidin-produced blister fluid is significantly lower than in serum. A study of ten subjects by Brehm (7) showed the total protein consistently lower in blister fluid with close correlation of the percentage values of the several fractions of the total protein. These studies, however, did not explore the possibility that the protein concentration of blister fluid might vary as a function of time following blister formation. Enzyme levels in blisters produced by cantharidin do vary with time and, with the exception of alkaline phosphatase, the enzyme values are much greater in blister fluid than serum (8).

The purpose of this study was to compare the total protein concentration of blister fluid with that of the blood serum at periodic time intervals beginning early in the blister's formation and extending to 48 hours after cantharidin application. Included is the evaluation and comparison of the several component fractions of the total protein from the serum and blister fluid at the specified times.

METHOD

Four blisters were raised on the upper arms of each of ten normal Caucasian subjects, age 18–26, previously screened to exclude those with histories of abnormalities such as atopy or unusual vascular reactions. The blisters were produced by 0.2 ml of 0.5% cantharidin (Inland Alkaloid Co.) in acetone applied inside a 15 mm rubber ring placed on the upper arm. The cantharidin solution was air dried, the ring removed, and a Sani-Fit® vaccination shield containing a small piece of slightly moistened cotton pad placed over the site. Just prior to the application of the cantharidin, a blood sample was taken, the serum separated from the clot, and frozen. At 7, 12, 24 and 48 hours after the application of cantharidin, a single blister was drained with a tuberculin syringe and the fluid frozen. Serum was also collected at these times and frozen. The total protein concentration of each of these samples was assayed by the method of Lowry et al (9). Commercial rabbit serum (Microbiological Assoc., Inc., Bethesda, Md.) of 7.3 g% total protein concentration was diluted 1:100 with normal saline and used as a standard. Determinations of optical density were obtained on a Coleman Jr. spectrophotometer at 650 mμ. The blister fluid and serum protein fractions were quantitated according to the procedure recommended by Beckman Company for use with the model R-101 microzone electrophoresis cell. The electrophoresis membranes were scanned with the model RB analytrol using the R-102 microzone scanning attachment.[1]

These studies supported in part by National Institutes of Health Grants 5T1 AM 5335 and AM 10522.

Received for publication April 7, 1967.

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RESULTS

Table 1 presents the mean values for the total protein in serum and in blister fluid at the various time intervals.

Significant differences are observed when values of blister fluid total protein are compared with those of serum. At no single measurement was there greater concentration of protein in the blister fluid than in the serum.

Table 2 gives the mean percentage of total protein that each of the several fractions occupies.

The percentage values of albumin, e.g., are seen to remain relatively consistent in both serum and blister fluid at the several time intervals and also closely concur. The α, β, and γ fractions are also essentially stable and are not significantly different in the two separate fluids.

TABLE 1

Mean values for total protein in serum and blister fluid

Time	Serum protein		Blister fluid protein		P
	No. tests	Mean ± S.D.	No. tests	Mean ± S.D.	
Initial	10	7.44 ± .56			
7 hrs	10	7.26 ± 1.60	4	5.29 ± .95	< .05
12 hrs	9	7.63 ± .51	7	5.47 ± .53	< .01
24 hrs	7	6.99 ± .78	8	5.84 ± 1.28	< .05
48 hrs	10	7.35 ± .41	8	5.80 ± .50	< .01

TABLE 2

Percent of protein fractions in serum and blister fluid

Time after application of cantharidin		Albumin	α_1	α_2	β_1	β_2	γ
		percent					
Serum	0 time	59.2	4.28	9.86	7.97	4.25	13.62
	7 hrs	58.9	4.50	10.70	7.67	3.68	14.36
	12 hrs	60.3	4.23	9.65	8.60	3.43	14.04
	24 hrs	63.0	4.10	8.70	9.27	3.72	11.94
	48 hrs	60.2	3.66	10.60	7.67	4.05	13.80
Blister fluid	7 hrs	50.17	5.25	12.2	10.10	5.86	16.4
	12 hrs	62.1	3.55	8.84	9.64	3.5	12.38
	24 hrs	63.3	4.19	8.72	9.06	3.24	11.43
	48 hrs	59.7	4.38	9.01	8.40	3.46	15.10

COMMENT

Evaluation of the data presented in Table 1 shows a significantly lower concentration of protein in cantharidin-induced blister fluid as compared to serum. The blister fluid values ranged from 70–85% of the serum protein values. The variation in the serum protein values at the several evaluations is considered minor and is attributed to the variations inherent in the measuring process. No consistent pattern can be established in the serum protein figures. It is unwise to attribute significance to the rise in blister fluid protein concentration to a maximum value at 24 hours since the changes are so small and these are mean values.

The various fractions comprise a rather constant percentage of the total protein in both blister fluid and serum. Consden and Smith (10) have suggested that there is a rapid turnover of serum proteins within the blisters, citing that there is no observable diminution of the labile β_2 globulin band in the blister fluid as compared with that of the sera, even in 40 hour old blisters. The increased capillary permeability occurring in inflammation would also lead one to suspect similarities in comparing proteins of cantharidin blister fluid with those of serum.

Studies by Lever (1) have demonstrated that any major abnormality in the electrophoretic pattern of the blood serum is also apparent in the pattern of the corresponding blister fluid. Seven electrophoretic analyses of serum and blister fluid protein were carried out by him in four patients with pemphigus vulgaris. The average values for the various proteins in blister fluid and blood serum were all quite similar and the hypoalbuminemia in his patients was reflected in a low value for albumin in the blister fluid.

In the normal volunteers of our study the albumin is in the 60% range both in serum and in blister fluid. These observations appear to support the concept that the blister fluid reflects the variation in serum protein and the several fractions.

SUMMARY

Fluid from intact cantharidin blisters was collected at 7, 12, 24 and 48 hours after application of the vesicant, cantharidin. Serum was

collected initially and concurrently with the blister fluid collection from the volunteers. Total protein and protein fraction evaluations were carried out in an effort to determine whether or not significant changes could be recognized as a function of time.

It was concluded that: 1. Total protein values were significantly lower in blister fluid than in serum at all four time intervals after cantharidin application. 2. Albumin and α_1, α_2, β_1, β_2 and γ globulins were found to account for specific percentages of the total protein, showed minimal variation with time, and reflected similar values in the two fluids.

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